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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Patricia Sarcabal *et al.*

Serial No.: 10/043,639

Filed: January 9, 2002

For: METHOD FOR PREPARING 1,3-
PROPANEDIOL BY A RECOMBINANT
MICRO-ORGANISM IN THE ABSENCE
OF COENZYME B12 OR ONE OF ITS
PRECURSORS

Group Art Unit: Unknown

Examiner: Unknown

Atty. Dkt. No.: CHEP:004US

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Mark B. Wilson

PRELIMINARY AMENDMENT

BOX MISSING PARTS
Commissioner for Patents
Washington, D.C. 20231

Sir:

Applicants respectfully submit this Preliminary Amendment in the above-referenced case.

Consideration of this case in view of the amendments made herein is respectfully requested.

AMENDMENT

In the Claims:

Please cancel claim 1 without prejudice or disclaimer.

Please add new claims 33-85 as follows:

33. (New) A process for the preparation of 1,3-propanediol from a carbon-containing substance comprising growing a recombinant micro-organism that comprises at least one nucleic acid coding for two sub-units of a glycerol dehydratase, wherein the catalytic activity of the glycerol dehydratase is not dependent on coenzyme B12 or one of its precursors.
34. (New) The process of claim 33, wherein the glycerol dehydratase is derived from *Clostridium butyricum*.
35. (New) The process of claim 33, wherein the glycerol dehydratase is a dimeric protein comprising:
 - (a) a first polypeptide having at least 50% amino acid identity with the amino acid sequence of SEQ ID NO. 6; and
 - (b) a second polypeptide having at least 50% amino acid identity with the amino acid sequence of SEQ ID NO. 7.
36. (New) The process of claim 33, wherein the recombinant micro-organism further comprises a 1,3-propanediol dehydrogenase.
37. (New) The process of claim 36, wherein the 1,3-propanediol dehydrogenase is derived from *Clostridium butyricum* VPI 1718.
38. (New) The process of claim 37, wherein the 1,3-propanediol dehydrogenase is a polypeptide having at least 90% amino acid identity with the amino acid sequence of SEQ ID NO. 8.
39. (New) The process of claim 33, wherein the recombinant micro-organism is grown in the absence of coenzyme B12 or one of its precursors.

40. (New) The process of claim 33, wherein the carbon-containing substance is a carbohydrate or polyol.
41. (New) The process of claim 40, wherein the carbon containing substance is glucose.
42. (New) The process of claim 40, wherein the carbon containing substance is glycerol.
43. (New) The process of claim 33, wherein the recombinant micro-organism does not naturally produce coenzyme B12 or one of its precursors.
44. (New) The process of claim 43, wherein the recombinant micro-organism is a bacterium, a yeast or a fungus.
45. (New) The process of claim 44, wherein the micro-organism is a bacterium.
46. (New) The process of claim 45, wherein the bacterium is a *Clostridium*, *Escherichia*, *Bacillus*, *Lactobacillus* or *Lactococcus* bacterium.
47. (New) The process of claim 44, wherein the recombinant micro-organism is yeast.
48. (New) The process of claim 47, wherein the yeast is *Saccharomyces cerevisiae*.
49. (New) The process of claim 33, wherein the recombinant micro-organism further comprises a nucleic acid coding for a glycerol-3-phosphate dehydrogenase and a nucleic acid coding for a glycerol-3-phosphatase.
50. (New) A recombinant nucleic acid coding for at least one subunit of a glycerol dehydratase, wherein the catalytic activity of the glycerol dehydratase is not dependent on coenzyme B12 or one of its precursors.

51. (New) The recombinant nucleic acid of claim 50, wherein the nucleic acid further encodes for two sub-units of the glycerol dehydratase.
52. (New) The recombinant nucleic acid of claim 50, wherein the nucleic acid comprises a polynucleotide region comprising at least 50% nucleotide identity with the nucleic acid sequences of SEQ ID NO. 1 or SEQ ID NO. 2, or a polynucleotide with a complementary sequence.
53. (New) The recombinant nucleic acid of claim 52, wherein the nucleic acid comprises:
- (a) a first polynucleotide region having at least 50% nucleotide identity with the nucleic acid sequence of SEQ ID NO. 1; and
 - (b) a second polynucleotide region having at least 50% nucleotide identity with the nucleic acid sequence of SEQ ID NO. 2.
54. (New) The recombinant nucleic acid of claim 53 further comprising a third polynucleotide region having at least 90% nucleotide identity with SEQ ID NO 4.
55. (New) The recombinant nucleic acid of claim 54, wherein SEQ ID NO. 1 and SEQ ID NO. 2 are positioned 5' to SEQ ID NO. 4.
56. (New) The recombinant nucleic acid of claim 54, wherein the nucleic acid comprises at least 50% nucleotide identity with the nucleic acid sequence of SEQ ID NO. 5.
57. (New) The recombinant nucleic acid of claim 54 further comprising fourth polynucleotide region coding for a glycerol-3-phosphate dehydrogenase and a fifth polynucleotide region coding for a glycerol-3-phosphatase.
58. (New) The recombinant nucleic acid of claim 53, wherein the nucleic acid further comprises a sequence with a transcription promoter function.

59. (New) The recombinant nucleic acid of claim 58, wherein the promoter sequence comprises at least 80% nucleotide identity with SEQ ID NO. 3.
60. (New) The recombinant nucleic acid of claim 58, wherein the promoter sequence comprises SEQ ID NO. 3.
61. (New) The recombinant nucleic acid of claim 50, further defined as comprised in a vector.
62. (New) The recombinant nucleic acid of claim 61, wherein the vector is further defined as an expression vector.
63. (New) The recombinant nucleic acid of claim 61, wherein the vector is further defined as a cloning vector.
64. (New) The recombinant nucleic acid of claim 61, wherein the vector is further defined as comprised in a host cell.
65. (New) The recombinant nucleic acid of claim 61, wherein the host cell is an *Escherichia coli* strain filed at the National Collection of Cultures of Micro-organisms (NCCM) on June 24, 1999 under the access No. I-2243.
66. (New) The recombinant nucleic of claim 61, wherein the vector is plasmid pSPD5.
67. (New) A recombinant nucleic acid sequence with a bacterial promoter function comprising a polynucleotide region having at least 80% nucleotide identity with the sequence SEQ ID NO. 3, or a polynucleotide with a complementary sequence.

68. (New) A recombinant nucleic acid comprising a first polynucleotide region coding for a 1,3-propanediol dehydrogenase comprising at least 90% nucleotide identity with SEQ ID NO.4.
69. (New) The recombinant nucleic acid of claim 68 further comprising a second polynucleotide region comprising at least 50% nucleotide identity with the nucleic acid sequences of SEQ ID NO. 1 or SEQ ID NO. 2.
70. (New) The recombinant nucleic acid of claim 69, wherein SEQ ID NO. 1 or SEQ ID NO. 2 are positioned 5' to SEQ ID NO. 4.
71. (New) The recombinant nucleic acid of claim 69, wherein the nucleic acid comprises at least 50% nucleotide identity with the nucleic acid sequence of SEQ ID NO. 5.
72. (New) The recombinant nucleic acid of claim 69, wherein the nucleic acid further comprises a third polynucleotide region coding for a glycerol-3-phosphate dehydrogenase and a fourth polynucleotide region coding for a glycerol-3-phosphatase.
73. (New) The recombinant nucleic acid of claim 68, further defined as comprised in a vector.
74. (New) The recombinant nucleic acid of claim 73, wherein the vector is further defined as an expression vector.
75. (New) The recombinant nucleic acid of claim 73, wherein the vector is further defined as a cloning vector.
76. (New) The recombinant nucleic acid of claim 73, wherein the vector is further defined as comprised in a host cell.

77. (New) The recombinant nucleic acid of claim 76, wherein the host cell is an *Escherichia coli* strain filed at the National Collection of Cultures of Micro-organisms (NCCM) on June 24, 1999 under the access No. I-2243.
78. (New) The expression vector of claim 73, wherein the vector is plasmid pSPD5.
79. (New) A polypeptide encoded by a recombinant nucleic acid coding for at least one subunit of a glycerol dehydratase, wherein the catalytic activity of the glycerol dehydratase is not dependent on coenzyme B12 or one of its precursors and wherein the polypeptide comprises at least 50% amino acid identity with the amino acid sequence of SEQ ID NO.6 or SEQ ID NO. 7.
80. (New) A dimeric protein comprising:
 - (a) a first polypeptide comprising at least 50% amino acid identity with the amino acid sequence of SEQ ID NO. 6; and
 - (b) a second polypeptide comprising at least 50% amino acid identity with the amino acid sequence of SEQ ID NO. 7.
81. (New) A polypeptide encoded by a recombinant nucleic acid comprising a first polynucleotide region coding for a 1,3-propanediol dehydrogenase comprising at least 90% nucleotide identity with SEQ ID NO. 4, wherein the polypeptide comprises an amino acid sequence of at least 90% amino acid identity with the amino acid sequence of SEQ ID NO. 8.
82. (New) A process for the production of a polypeptide encoded by a recombinant nucleic acid coding for at least one subunit of a glycerol dehydratase, wherein the catalytic activity of the glycerol dehydratase is not dependent on coenzyme B12 and wherein the polypeptide comprises at least 50% amino acid identity with the amino acid sequence of SEQ ID NO. 6 or SEQ ID NO. 7, or a dimeric protein comprising a first polypeptide comprising at least 50% amino acid identity with the amino acid sequence of SEQ ID

NO. 6 and a second polypeptide comprising at least 50% amino acid identity with the amino acid sequence of SEQ ID NO. 7 or a polypeptide encoded by a recombinant nucleic acid comprising a first polynucleotide region coding for a 1,3-propanediol dehydrogenase comprising at least 90% nucleotide identity with SEQ ID NO. 4, wherein the polypeptide comprises an amino acid sequence of at least 90% amino acid identity with the amino acid sequence of SEQ ID NO. 8 comprising:

- (a) preparation of an expression vector;
 - (b) introduction of the expression vector into a host cell;
 - (c) culture of the host cell in a suitable medium; and
 - (d) recovery of the polypeptide produced from the host cell,
83. (New) The process of claim 82 further comprising purifying the polypeptide produced from the host cell.
84. (New) The process of claim 82, wherein the polypeptide is recovered from the culture supernatant or the cell lysate.
85. (New) An antibody directed against a polypeptide encoded by a recombinant nucleic acid coding for at least one subunit of a glycerol dehydratase, wherein the catalytic activity of the glycerol dehydratase is not dependent on coenzyme B12 and wherein the polypeptide comprises at least 50% amino acid identity with the amino acid sequence of SEQ ID NO. 6 or SEQ ID NO. 7, or a dimeric protein comprising a first polypeptide comprising at least 50% amino acid identity with the amino acid sequence of SEQ ID NO. 6 and a second polypeptide comprising at least 50% amino acid identity with the amino acid sequence of SEQ ID NO. 7 or a polypeptide encoded by a recombinant nucleic acid comprising a first polynucleotide region coding for a 1,3-propanediol dehydrogenase comprising at least 90% nucleotide identity with SEQ ID NO. 4, wherein the polypeptide comprises an amino acid sequence of at least 90% amino acid identity with the amino acid sequence of SEQ ID NO. 8.--

REMARKS

Claim 1 has been cancelled without prejudice or disclaimer and claims 33-85 have been added. Support for these claims may be found throughout the specification. Therefore, claims 33-85 are presently pending. A clean copy of the presently pending claims is attached as Appendix A.

Should any additional fees under 37 C.F.R. §§ 1.16 to 1.21 be required, the Commissioner is hereby authorized to deduct said fees from Fulbright & Jaworski Deposit Account No. 50-1212/CHEP:004US.

Respectfully submitted,



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APPENDIX A
Presently Pending Claims

33. A process for the preparation of 1,3-propanediol from a carbon-containing substance comprising growing a recombinant micro-organism that comprises at least one nucleic acid coding for two sub-units of a glycerol dehydratase, wherein the catalytic activity of the glycerol dehydratase is not dependent on coenzyme B12 or one of its precursors.
34. The process of claim 33, wherein the glycerol dehydratase is derived from *Clostridium butyricum*.
35. The process of claim 33, wherein the glycerol dehydratase is a dimeric protein comprising:
 - (a) a first polypeptide having at least 50% amino acid identity with the amino acid sequence of SEQ ID NO. 6; and
 - (b) a second polypeptide having at least 50% amino acid identity with the amino acid sequence of SEQ ID NO. 7.
36. The process of claim 33, wherein the recombinant micro-organism further comprises a 1,3-propanediol dehydrogenase.
37. The process of claim 36, wherein the 1,3-propanediol dehydrogenase is derived from *Clostridium butyricum* VPI 1718.
38. The process of claim 37, wherein the 1,3-propanediol dehydrogenase is a polypeptide having at least 90% amino acid identity with the amino acid sequence of SEQ ID NO. 8.
39. The process of claim 33, wherein the recombinant micro-organism is grown in the absence of coenzyme B12 or one of its precursors.

40. The process of claim 33, wherein the carbon-containing substance is a carbohydrate or polyol.
41. The process of claim 40, wherein the carbon containing substance is glucose.
42. The process of claim 40, wherein the carbon containing substance is glycerol.
43. The process of claim 33, wherein the recombinant micro-organism does not naturally produce coenzyme B12 or one of its precursors.
44. The process of claim 43, wherein the recombinant micro-organism is a bacterium, a yeast or a fungus.
45. The process of claim 44, wherein the micro-organism is a bacterium.
46. The process of claim 45, wherein the bacterium is a *Clostridium*, *Escherichia*, *Bacillus*, *Lactobacillus* or *Lactococcus* bacterium.
47. The process of claim 44, wherein the recombinant micro-organism is yeast.
48. The process of claim 47, wherein the yeast is *Saccharomyces cerevisiae*.
49. The process of claim 33, wherein the recombinant micro-organism further comprises a nucleic acid coding for a glycerol-3-phosphate dehydrogenase and a nucleic acid coding for a glycerol-3-phosphatase.
50. A recombinant nucleic acid coding for at least one subunit of a glycerol dehydratase, wherein the catalytic activity of the glycerol dehydratase is not dependent on coenzyme B12 or one of its precursors.

51. The recombinant nucleic acid of claim 50, wherein the nucleic acid further encodes for two sub-units of the glycerol dehydratase.
52. The recombinant nucleic acid of claim 50, wherein the nucleic acid comprises a polynucleotide region comprising at least 50% nucleotide identity with the nucleic acid sequences of SEQ ID NO. 1 or SEQ ID NO. 2, or a polynucleotide with a complementary sequence.
53. The recombinant nucleic acid of claim 52, wherein the nucleic acid comprises:
- (a) a first polynucleotide region having at least 50% nucleotide identity with the nucleic acid sequence of SEQ ID NO. 1; and
 - (b) a second polynucleotide region having at least 50% nucleotide identity with the nucleic acid sequence of SEQ ID NO. 2.
54. The recombinant nucleic acid of claim 53 further comprising a third polynucleotide region having at least 90% nucleotide identity with SEQ ID NO 4.
55. The recombinant nucleic acid of claim 54, wherein SEQ ID NO. 1 and SEQ ID NO. 2 are positioned 5' to SEQ ID NO. 4.
56. The recombinant nucleic acid of claim 54, wherein the nucleic acid comprises at least 50% nucleotide identity with the nucleic acid sequence of SEQ ID NO. 5.
57. The recombinant nucleic acid of claim 54 further comprising fourth polynucleotide region coding for a glycerol-3-phosphate dehydrogenase and a fifth polynucleotide region coding for a glycerol-3-phosphatase.
58. The recombinant nucleic acid of claim 53, wherein the nucleic acid further comprises a sequence with a transcription promoter function.

59. The recombinant nucleic acid of claim 58, wherein the promoter sequence comprises at least 80% nucleotide identity with SEQ ID NO. 3.
60. The recombinant nucleic acid of claim 58, wherein the promoter sequence comprises SEQ ID NO. 3.
61. The recombinant nucleic acid of claim 50, further defined as comprised in a vector.
62. The recombinant nucleic acid of claim 61, wherein the vector is further defined as an expression vector.
63. The recombinant nucleic acid of claim 61, wherein the vector is further defined as a cloning vector.
64. The recombinant nucleic acid of claim 61, wherein the vector is further defined as comprised in a host cell.
65. The recombinant nucleic acid of claim 61, wherein the host cell is an *Escherichia coli* strain filed at the National Collection of Cultures of Micro-organisms (NCCM) on June 24, 1999 under the access No. I-2243.
66. The recombinant nucleic acid of claim 61, wherein the vector is plasmid pSPD5.
67. A recombinant nucleic acid sequence with a bacterial promoter function comprising a polynucleotide region having at least 80% nucleotide identity with the sequence SEQ ID NO. 3, or a polynucleotide with a complementary sequence.
68. A recombinant nucleic acid comprising a first polynucleotide region coding for a 1,3-propanediol dehydrogenase comprising at least 90% nucleotide identity with SEQ ID NO.4.

69. The recombinant nucleic acid of claim 68 further comprising a second polynucleotide region comprising at least 50% nucleotide identity with the nucleic acid sequences of SEQ ID NO. 1 or SEQ ID NO. 2.
70. The recombinant nucleic acid of claim 69, wherein SEQ ID NO. 1 or SEQ ID NO. 2 are positioned 5' to SEQ ID NO. 4.
71. The recombinant nucleic acid of claim 69, wherein the nucleic acid comprises at least 50% nucleotide identity with the nucleic acid sequence of SEQ ID NO. 5.
72. The recombinant nucleic acid of claim 69, wherein the nucleic acid further comprises a third polynucleotide region coding for a glycerol-3-phosphate dehydrogenase and a fourth polynucleotide region coding for a glycerol-3-phosphatase.
73. The recombinant nucleic acid of claim 68, further defined as comprised in a vector.
74. The recombinant nucleic acid of claim 73, wherein the vector is further defined as an expression vector.
75. The recombinant nucleic acid of claim 73, wherein the vector is further defined as a cloning vector.
76. The recombinant nucleic acid of claim 73, wherein the vector is further defined as comprised in a host cell.
77. The recombinant nucleic acid of claim 76, wherein the host cell is an *Escherichia coli* strain filed at the National Collection of Cultures of Micro-organisms (NCCM) on June 24, 1999 under the access No. I-2243.

78. The expression vector of claim 73, wherein the vector is plasmid pSPD5.
79. A polypeptide encoded by a recombinant nucleic acid coding for at least one subunit of a glycerol dehydratase, wherein the catalytic activity of the glycerol dehydratase is not dependent on coenzyme B12 or one of its precursors and wherein the polypeptide comprises at least 50% amino acid identity with the amino acid sequence of SEQ ID NO.6 or SEQ ID NO. 7.
80. A dimeric protein comprising:
- (a) a first polypeptide comprising at least 50% amino acid identity with the amino acid sequence of SEQ ID NO. 6; and
 - (b) a second polypeptide comprising at least 50% amino acid identity with the amino acid sequence of SEQ ID NO. 7.
81. A polypeptide encoded by a recombinant nucleic acid comprising a first polynucleotide region coding for a 1,3-propanediol dehydrogenase comprising at least 90% nucleotide identity with SEQ ID NO. 4, wherein the polypeptide comprises an amino acid sequence of at least 90% amino acid identity with the amino acid sequence of SEQ ID NO. 8.
82. A process for the production of a polypeptide encoded by a recombinant nucleic acid coding for at least one subunit of a glycerol dehydratase, wherein the catalytic activity of the glycerol dehydratase is not dependent on coenzyme B12 and wherein the polypeptide comprises at least 50% amino acid identity with the amino acid sequence of SEQ ID NO. 6 or SEQ ID NO. 7, or a dimeric protein comprising a first polypeptide comprising at least 50% amino acid identity with the amino acid sequence of SEQ ID NO. 6 and a second polypeptide comprising at least 50% amino acid identity with the amino acid sequence of SEQ ID NO. 7 or a polypeptide encoded by a recombinant nucleic acid comprising a first polynucleotide region coding for a 1,3-propanediol dehydrogenase comprising at least 90% nucleotide identity with SEQ ID NO. 4, wherein the polypeptide

comprises an amino acid sequence of at least 90% amino acid identity with the amino acid sequence of SEQ ID NO. 8 comprising:

- (a) preparation of an expression vector;
- (b) introduction of the expression vector into a host cell;
- (c) culture of the host cell in a suitable medium; and
- (d) recovery of the polypeptide produced from the host cell,

83. The process of claim 82 further comprising purifying the polypeptide produced from the host cell.
84. The process of claim 82, wherein the polypeptide is recovered from the culture supernatant or the cell lysate.
85. An antibody directed against a polypeptide encoded by a recombinant nucleic acid coding for at least one subunit of a glycerol dehydratase, wherein the catalytic activity of the glycerol dehydratase is not dependent on coenzyme B12 and wherein the polypeptide comprises at least 50% amino acid identity with the amino acid sequence of SEQ ID NO. 6 or SEQ ID NO. 7, or a dimeric protein comprising a first polypeptide comprising at least 50% amino acid identity with the amino acid sequence of SEQ ID NO. 6 and a second polypeptide comprising at least 50% amino acid identity with the amino acid sequence of SEQ ID NO. 7 or a polypeptide encoded by a recombinant nucleic acid comprising a first polynucleotide region coding for a 1,3-propanediol dehydrogenase comprising at least 90% nucleotide identity with SEQ ID NO. 4, wherein the polypeptide comprises an amino acid sequence of at least 90% amino acid identity with the amino acid sequence of SEQ ID NO. 8.